



## The Newsletter of the Center for Coastal Environmental Health & Biomolecular Research

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# The National Biomonitoring Specimen Bank

by Rebecca S. Pugh

The National Institute of Standards and Technology (NIST) has been involved in long term environmental specimen banking for 20 years. The U.S. National Biomonitoring Specimen Bank (NBSB) was developed in 1979 from a pilot Environmental Specimen Bank Program sponsored by the Environmental Protection Agency (EPA) (Wise and Zeisler, 1985). The NBSB is maintained at NIST in Gaithersburg, Maryland. The NBSB is a specimen banking system that provides for the long-term storage of well documented and cryogenically preserved environmental specimens. Specimen banking provides researchers the ability to look at environmental trends over long periods of time, conduct intercomparison exercises with other laboratories as part of monitoring programs, and provides valuable baseline data which is currently limited.



*Samples are stored in cardboard tubes  
located in liquid nitrogen*

In 1995, an agreement was signed between NIST and NOAA's National Marine Fisheries Service (NMFS) to establish the National Marine Analytical Quality Assurance Program (NMAQAP). As part of this agreement, NIST established a satellite of the NBSB at CCEHBR in Charleston, South Carolina. Through interlaboratory comparisons and reference material development, the NMAQAP will be able to assess and improve the quality of analytical measurements in the marine environment as well as improve the capabilities to assess trends in marine environmental quality by expanding environmental specimen

banking activities. The NBSB-Charleston Laboratory is dedicated to banking only marine specimens. Currently, there are three programs that contribute to the NBSB-Charleston; the Alaska Marine Mammal Tissue Archival Project (AMMTAP), the Marine Mammal Health and Stranding Response Program (MMHSRP) and the Seabird Tissue Archival and Monitoring Project (STAMP).

The AMMTAP was initiated in 1987 with sponsorship from the U.S. Department of Interior, Minerals Management Service (MMS). The project is now conducted as a collaboration between U.S. Geological Survey/Biological Resources Division (USGS/BRD) and NIST. The goals of this program are to establish a representative collection of marine mammal tissues taken during Alaska Native subsistence hunts for future contaminant analyses and documentation of long-term trends in environmental quality (Zeisler, et al., 1992). A detailed description of the project and protocols have been published (Becker, et al., 1991; 1993).

The National Marine Mammal Tissue Bank (NMMTB) was established in 1987 by NMFS to collect and archive marine mammal tissues at the NBSB. In 1992, the NMMTB was formally established by Federal Legislation (Public Law 102-587) and a portion of tissues collected by NMFS's MMHSRP are archived in the NMMTB. The MMHSRP obtains specimens from marine mammal strandings and from animals taken incidentally during commercial fishing operations, and emphasizes contaminant monitoring and information management. Detailed descriptions of this project have been published (Lillestolen, et al. 1993; Becker, et al. 1994).

The third program that currently contributes to the NBSB is the STAMP. This program was initiated in 1998 with financial support from the U.S. Department of Interior, USGS Biological Resources Division (USGS/BRD) and is conducted as a collaboration among USGS/BRD, U.S. Fish and Wildlife Service Alaska Maritime National Wildlife Refuge, and NIST. This program archives collections of seabird tissues from representative Alaskan colonial seabird species for future contaminant analyses and documentation of long-term trends in environmental quality. Currently, seabird eggs from common murre (*Uria aalge*) and thick-billed murre (*U. lomvia*) colonies are collected and banked but additional types of species and specimens will be collected in the future. A description of this project is presented in York et al.(in press).

The NBSB-Charleston has been designed to contain a Class 10,000 cryogenic storage room (Specimen Bank-Room 227A) and a Class 100 sample processing area (Clean Room-Room 227B). Laboratory frocks, hoods, and shoe covers must be worn at all times while in the NBSB to prevent further contamination of samples. The garments are made of non-woven Tyvek, a highly effective contamination control fabric that is resistant to penetration by airborne particles. Samples that are collected for AMMTAP, MMHSRP, and STAMP are maintained in the bank through the Archival Specimen Tracking Retrieval Operation (ASTRO) database. ASTRO manages three major processes: Specimen Management, Storage Management, and System Administration. These samples are archived in liquid nitrogen vapor phase (-150 degrees C) freezers that are located in the Specimen Bank. Two 150 g portions of liver, kidney and blubber are collected from a list of target species and labeled A and B. Portion A is for permanent storage in the Specimen Bank while portion B is available for analyses. When selected for analyses, Portion B is prepared by cryogenic homogenization. Cryogenic conditions are maintained throughout the homogenizing process to reduce the potential loss of volatile compounds and avoid sample degradation due to thawing and re-freezing. This process is conducted in the Clean Room along with the preparation and cleaning of Teflon materials used for homogenizing, and Teflon jars and titanium knives used for field collections.

In February 1999, the NBSB-Charleston Laboratory began receiving and archiving all samples that were

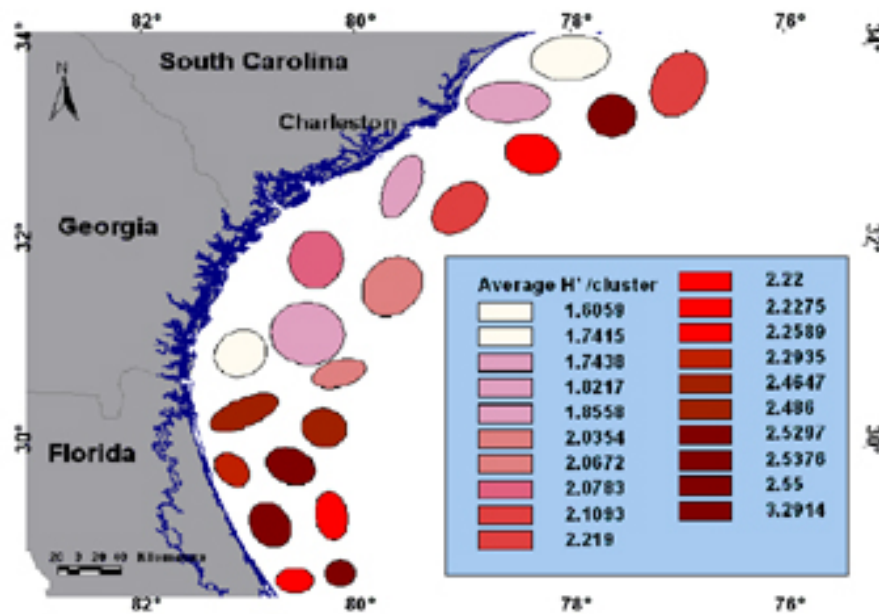


# NOS/Sanctuaries Joint Issues: Spatial statistics and analysis for defining regions of high biological diversity based on long-term fisheries data.

by Laura Kracker and Jill Jennings

Efforts to establish and manage marine protected areas have focused on ecologically significant resources and consideration of stakeholder input (eg. Channel Islands Demonstration Project involving NMFS, Coastal Service Center, National Park Service and other stakeholders). New spatial statistical techniques applied to long-term fisheries data can provide additional information to improve the decision making and management processes. In this context, research conducted at CCEHBR provides support in addressing joint NOS/Sanctuaries issues.

At CCEHBR, spatial statistical techniques are being applied to long-term fisheries-independent data to identify areas of high biological diversity in the South Atlantic Bight (SAB). The advantage of this approach is that it utilizes long-term fisheries information to differentiate specific geographic locations that are unique in supporting biological diversity.



K-means clustering is used to differentiate geographic regions based on the Shannon-Weiner index of biological diversity ( $H'$ ) for pelagic finfish. Diversity indices were derived from MARMAP trawl data and then clustered for locations throughout the SAB. Clusters are defined such that variance in  $H'$  is minimized, grouping sites that are similar in biological diversity. The differences between classes or clusters is seen by assigning the average  $H'$  to each cluster. Dark red ellipses indicate areas of higher diversity.

These techniques help differentiate biologically diverse areas. Further analysis using Principal Components Analysis links diversity measures with environmental parameters to better understand habitat

conditions that support pelagic species.

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## Risk Management of Pathogenic *Vibrio vulnificus* in Molluscan Shellfish

by Paul Comar and Jan Gooch-Moore

Several species of bacteria belonging to the genus *Vibrio* and capable of causing human illness occur naturally in coastal waters. One of these, *V. vulnificus*, is particularly troublesome. Although this pathogen has been isolated from all U.S. coastal waters, *V. vulnificus* densities are highest in warmer waters with low to moderate salinities, mainly the Gulf of Mexico. Through their filter feeding mechanisms, oysters and other bivalve shellfish bioaccumulate bacteria, including *V. vulnificus*, to levels sometimes 100 times that of the overlying water.

*V. vulnificus* illnesses, ranging from gastroenteritis to acute septicemia, are relatively rare in the U.S. and are caused almost entirely by wound infections or consumption of raw oysters from the Gulf of Mexico. While illness incidence is very low, its severity and associated high mortality are of great concern to public health authorities and the shellfish industry. From 1989 through 1998, an average of 20 cases and 10 deaths occurred annually. Healthy individuals are at very low risk, but certain health factors increase the risk of infection. Nearly 75% of cases resulting in primary septicemia occur in persons with liver disease or heavy alcohol intake. Other risk factors include hemochromatosis (an iron storage disease), diabetes, cancer, and immune system disorders.

The presence and densities of *V. vulnificus* are not related to fecal pollution of shellfish growing waters, thus current harvesting regulations do not serve to prevent these illnesses. Federal and state health authorities along with the shellfish industry through the Interstate Shellfish Sanitation Conference (ISSC) are cooperatively developing and implementing several strategies to reduce the illness risk from shellfish consumption. The focus is on education and risk communication to shellfish consumers in the medically-compromised groups, improved post harvest shellfish handling practices, shellfish processing to reduce vibrios in shellfish to non-detectable levels, and research on *V. vulnificus* and factors affecting its virulence.



CCEHBR is an active participant in developing and evaluating these risk management strategies through



the ISSC with state shellfish control agencies, the FDA, EPA and shellfish industry. Center scientists serve in key positions to develop both the education plan and its implementation strategy through NOAA funding. Additional funding support may be made available in 2001 from Sea Grant and the EPA to address portions of the overall risk communication plan. Among other tasks this year, targeted risk messages will be delivered through health advocacy groups, e.g. the American Liver Foundation, and through state associations in six states which account for 80% of illness reports. Measures will be made of the effectiveness both in delivery of the message and its impact in changing behavior of those at risk to avoid raw oyster consumption.

CCEHBR also serves on the ISSC's *Vibrio* Management Committee which is finalizing a comprehensive risk management approach for both *V. vulnificus* and *V. parahaemolyticus* (another common disease agent) illnesses associated with raw shellfish consumption. One critical aspect of the *V. vulnificus* plan is the adoption of a performance standard for disease mitigation, and CCEHBR has provided much baseline data on the standard's formula, its factors and reduction targets. A six year management plan is being established as three biennial segments with goals, tasks, performance measures, assessments, and annual reports.

NOAA is represented by CCEHBR on an advisory team which recommends funding on extra-mural *V. vulnificus* research. Through this mechanism, over \$1 million dollars have been targeted in recent years to scientific investigations of environmental, shellfish handling, and virulence factors affecting the prevalence and pathogenicity of *V. vulnificus*. Additionally, one recent study through CCEHBR determined growth and survival of *V. vulnificus* in post harvest Gulf Coast oysters held at 26 degrees C for 0, 5, 10, and 24hr. After each time interval the oysters were refrigerated at 3 degrees C and analyzed two weeks later. Results showed that decreasing the amount of time prior to refrigeration reduces vibrio multiplication and may lower human health risk by lowering the dose consumed.

The combination of shellfish handling, processing, risk communication and research approaches are designed to satisfy consumer health concerns and the shellfish industry's interest that regulations not be overly restrictive. Scheduled reassessments of results in each of these areas ensure effective use of resources and improved control practices to better manage risk.

## Upcoming Events

Fort Johnson Marine Science Seminar Series - View the list of speakers at [http://www.chbr.noaa.gov/FTJSS\\_Spring01.html](http://www.chbr.noaa.gov/FTJSS_Spring01.html)

February 1 - Ecological Forecasting: Dr. Donald Scavia - NOS Chief Scientist (held in the CCEHBR Auditorium at 10:00 AM)

